Selective Antagonism of Central Nervous System Depressants by DH-524; A Comparative Study with Bemegride (37576)

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Widespread use and abuse of barbiturates has resulted in numerous cases of barbiturate poisoning. Usually victims of barbiturate overdose are given supportive care while diuresis, dialysis, and gastrointestinal adsorption are used to lower barbiturate blood levels. Unfortunately, there is no specific antagonist for use in barbiturate poisoning, although some investigators have advocated the use of powerful analeptic agents (1, 2) for shortening narcosis. Present knowledge views the antagonistic action of analeptics as the result of nonspecific central nervous system stimulation. In deep coma the difference between the lowest dose of analeptic producing a discernible effect and that producing convulsions may be very small (3). Also, at high doses the analeptics often produce vomiting and cardiac arrhythmias (5, 6).

This report deals with 2(3,4-dichlorophenoxy)methyl-2-imidazoline (DH-524),[1] an agent which shares with known analeptics the property of dramatically shortening barbiturate narcosis.[2] However, several important differences between DH-524 and a typical analeptic, bemegride, were discovered in these studies.

Materials and Methods. Male albino mice (Spartan strain Swiss Webster) from Spartan Research Animals, Inc. (Haslett, MI) were used in all studies. Animals of approximately the same age and weight were used in each experiment. Sleeping times were defined as the time from the ip injection of depressant until the animal was able to right himself twice. In each experiment narcosis was allowed to develop for 10 min after injection of the depressant, and then iv injections of Krebs buffer or test drugs dissolved in Krebs were given. Animals which were not asleep at the time of iv injection were eliminated from the experiment. Because there was considerable day to day variation in sleeping times, each experiment contained a control group which received only depressant and iv Krebs. Drug groups were then compared to their own controls by use of Student's t test.

Spontaneous motor activity measurements were obtained from individual animals with an electronic activity monitor (Stoelting Co.). Mice were placed in plastic cages (11 × 13 × 8 cm) for a 30 min acclimitization period during which no measurements were made. Subsequently pretreatment activity was recorded for 30 min and then drugs were administered. Recording of activity was continued for three 30 min periods. Since these experiments extended over a period of days in order to accumulate sufficient data, the effect of diurnal variation was distributed equally among the treatment groups (10 mice/group) by simultaneously testing one member from each group at each session.

Convulsant activity was determined by continuous iv infusion of 5 mg/ml solutions of drug. Ten mice were used for each group, with a weight range of 27–34 g. All animals received the drug solutions at a rate of 0.103 cc/min through a cannula implanted in the lateral tail vein. Animals were free to move about in a clear plastic enclosure (28 × 28 × 15 cm) during infusion. At the first appearance of convulsions, the infusion was terminated and the time to convulsion recorded. Animals were observed for a short

[1] The details of the preparation and purification of the DH-524 used in this study are reported in U.S. Pat. No. 3449355.

[2] Unpublished data, A. D. Rudzik and J. H. Mennear in this laboratory.

time following convulsion to determine whether the convulsion was terminal or not.

Bemegride was obtained as a commercial intravenous preparation (5 mg/ml, Abbott Laboratories) as was doxapram (20 mg/ml, A. H. Robbins). Dilutions of these preparations were made with saline. Methaqualone and glutethimide were gifts of the William H. Rorer and Ciba companies, respectively, and were administered as suspensions in 0.5% Methocel HG (The Dow Chemical Co.). Methoxyfluorane was obtained from The Dow Chemical Co., and chlorpromazine was a gift from Smith, Kline and French Co. Concentrations of the solutions or suspensions were adjusted so that the animals always received a volume of 10 ml/kg except in the case of methoxyfluorane which was administered at the rate of 1 ml/kg.

Results. An ip dose of 70 mg/kg of sodium pentobarbital consistently induced deep narcosis in our strain of mice. This dose was chosen for this study because lower doses (35 mg/kg, 50 mg/kg) produced inconsistent narcosis, while higher doses produced inconveniently long sleeping times (100 mg/kg) or death (150 mg/kg). As shown in Table I, iv doses of DH-524 from 5 mg/kg to 20 mg/kg significantly shortened sleeping times. (The iv LD_{50} of DH-524 is 21.5 mg/kg). Bemegride was also capable of shortening pentobarbital sleeping times. We found it caused significant shortening at a dose of 50 mg/kg, iv. This is in good agreement with the work of Kimura and Richards (4) who reported bemegride antagonized narcosis produced by 80 mg/kg ip of pentobarbital at a dose of 100 mg/kg. These authors also found that narcosis produced by 60 mg/kg ip of pentobarbital was antagonized by 10 mg/kg iv of bemegride. Short periods of convulsions were noted in the bemegride-treated animals but were not seen with the DH-524-treated groups.

Similarly, both DH-524 and bemegride were effective in shortening the narcosis induced by 100 mg/kg ip of methaqualone (Table I). DH-524 was effective in the 5–15 mg/kg iv range, while bemegride was effective at doses of 12.5, 25 and 50 mg/kg iv.

However, when narcosis was produced by the ip injection of either 200 mg/kg of glutethimide or 1 ml/kg (1.4 g/kg) of methoxyfluorane, a dramatic difference between the action of DH-524 and bemegride was noted. DH-524 produced, if anything, a slight lengthening of narcosis caused by these agents, while bemegride significantly shortened narcosis (Table I). Thus DH-524 is definitely more selective in its action than bemegride.

Although fatal doses of many types of drugs produce convulsions in mice (perhaps due to central nervous system hypoxia), it is rare to see convulsions with non-fatal doses. When bemegride and doxapram were given by intravenous infusion, tremor and general clonic convulsions were seen at mean doses of 13.3 ± 1.4 mg/kg and 113 ± 24 mg/kg, respectively. Infusion was terminated immediately upon observation of convulsions, and all of the animals receiving doxapram (10) and 8 of 10 animals receiving bemegride subsequently recovered. In contrast, no *nonfatal* dose of DH-524 produced convulsions. It was necessary to infuse a mean dose of 37.4 ± 4.4 mg/kg of DH-524 before any convulsions were observed. This dose is almost twice the LD_{50} for DH-524 (21.5 mg/kg by intravenous injection) and invariably proved fatal to our mice. These results suggest that, unlike bemegride and doxapram, DH-524 is not primarily a convulsant drug.

Spontaneous motor activity measurements were carried out upon groups of 10 animals dosed ip with saline or DH-524 dissolved in saline.

Mean activity counts for the four groups, plus or minus standard deviations, were: saline, 3560 ± 4660; DH-524 (10 mg/kg), 2970 ± 2030; DH-524 (22 mg/kg), 2640 ± 1050; DH-524 (46 mg/kg), 5090 ± 8650.

In the doses used, DH-524 did not have a significant effect upon spontaneous motor activity. (Results were analyzed by the Mann Whitney *U* test with the significance level set at 0.05).

Discussion. DH-524 shares with bemegride and other analeptics and stimulants the property of shortening narcosis in pentobarbital and methaqualone-treated mice. However,

TABLE I. Antagonism of Central Nervous System Depressants by DH-524 and Bemegride.[a]

Depressant	Expt no.	Control	Sleeping time (min) ± SD: Antagonists: DH-524 (mg/kg) 5	10	15	20	Bemegride (mg/kg) 12.5	25	50
Pentobarbital (70 mg/kg)	1	146 ± 7	—	—	—	125 ± 22[b] ($n = 8$)	—	—	—
	2	93 ± 21	—	67 ± 27[b]	—	90 ± 24	—	—	—
	3	143 ± 43	100 ± 48[b]	88 ± 25[c]	—	—	—	—	—
	4	79 ± 17	—	55 ± 15[c]	—	—	—	—	—
	5	80 ± 34	—	—	—	—	—	55 ± 29 ($n = 9$)	46 ± 23[b] ($n = 8$)
Glutethimide (200 mg/kg)	6	229 ± 99 ($n = 7$)	—	262 ± 123 ($n = 6$)	204 ± 106 ($n = 5$)	188 ± 82 ($n = 7$)	—	—	—
	7	158 ± 42 ($n = 11$)	194 ± 78	235 ± 128 ($n = 9$)	—	—	—	—	—
	8	346 ± 84	—	—	—	—	298 ± 117	204 ± 129[c]	150 ± 124[d]
Methoxyfluorane (1400 mg/kg)	9	135 ± 71 ($n = 8$)	153 ± 100 ($n = 6$)	206 ± 119 ($n = 5$)	219 ± 59 ($n = 3$)	—	—	—	—
	10	134 ± 20 ($n = 5$)	—	—	—	—	74 ± 22[d] ($n = 6$)	62 ± 23[d] ($n = 5$)	41 ± 8[d] ($n = 8$)
Methaqualone (100 mg/kg)	11	127 ± 67 ($n = 8$)	132 ± 55 ($n = 9$)	79 ± 62 ($n = 8$)	65 ± 63[b]	—	—	—	—
	12	138 ± 38	82 ± 28[c]	52 ± 39[c] ($n = 9$)	54 ± 34[c] ($n = 9$)	—	—	—	—
	13	218 ± 35	—	—	—	—	91 ± 83[c] ($n = 9$)	85 ± 110[c] ($n = 9$)	20 ± 13[c] ($n = 9$)

[a] Animals all received an intraperitoneal dose of the CNS depressants. Ten minutes later, they received either Krebs or Krebs solutions of the antagonists intravenously. Animals who were not asleep at the time of the intravenous injection were eliminated from the experiment. Sleeping times were defined as the time from the intraperitoneal injection of depressant until the animals regained the righting reflex. Ten animals were used in each experimental group except as noted.

[b] Significantly different than control group by Student's t test: ($p < 0.05$); [c] ($p < 0.01$); [d] ($p < 0.001$).

during the course of these studies we noted several important differences between the action of DH-524 and bemegride:

1. Narcotized animals receiving bemegride frequently had short periods of convulsions, particularly after the 50 mg/kg iv dose. None of the animals receiving DH-524 (at 5, 10, 15 or 20 mg/kg iv) exhibited this behavior.

2. Kimura and Richards (4) reported that with 80 mg/kg ip of pentobarbital in mice, doses of bemegride below 100 mg/kg iv were generally ineffective. As shown in Table I, with 70 mg/kg ip of pentobarbital, 50 mg/kg iv of bemegride significantly shortened sleeping times, but 25 mg/kg iv did not. However, intravenous doses of bemegride of 25 mg/kg or higher produced instant convulsions and death in non-narcotized mice. In contrast, the effective doses of DH-524 (from 5 to 20 mg/kg iv) were well tolerated by either narcotized or untreated animals.

3. Bemegride antagonized central depression, at least as measured by sleeping times, regardless of what agent was used to produce the depression. DH-524 was effective in shortening narcosis induced by pentobarbital and methaqualone, but did not shorten narcosis induced by glutethimide or methoxyfluorane. Preliminary studies in our lab have also shown that DH-524 is ineffective in reducing depression caused by chloropromazine, but does reduce depression caused by ethanol. DH-524 is therefore definitely more selective in its action than bemegride.

4. Nonfatal doses of both doxapram and bemegride were capable of inducing convulsions when infused in non-narcotized animals. In contrast, convulsions were not observed with any nonfatal dose of DH-524. Fatal doses of DH-524 did produce convulsions, but these may be due to hypoxia rather than a specific convulsant action.

It is also noteworthy that DH-524 can antagonize the effects of CNS depressants without itself producing an amphetamine-like stimulation of spontaneous motor activity.

Summary. DH-524 is an agent which antagonizes the effects of some central nervous system depressants, but is without effect upon the narcosis produced by other depressants. This differentiates it from analeptics and stimulants which are nonspecific in their action. DH-524 is further differentiated by its failure to induce convulsions at nonfatal doses and its lack of effect upon spontaneous motor activity. In addition, DH-524 is an effective antagonist at doses which are well tolerated by nonnarcotized animals, while effective doses of bemegride were very toxic to nonnarcotized animals. These data suggest that DH-524 would be more selective and have a wider margin of safety than the commonly used analeptics in the treatment of barbiturate or methaqualone overdose.

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